

Cynoglossum officinale Toxicity in Calves

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Summary

Six calves were given dried, ground *Cynoglossum officinale* daily in a dose which provided 15 (two calves) or 60 (four calves) mg per kg per day of total pyrrolizidine alkaloids. Those calves given 60 mg per kg of total pyrrolizidine alkaloids per day died following a single dose of plant material. These calves had a marked elevation of serum gamma-glutamyltransferase (GGT) and aspartate transaminase (AST) activities and serum bile acid and total bilirubin (TBili) concentrations. These four calves all had massive hepatocellular necrosis and haemorrhage of the liver. Of the two calves that were given 15 mg per kg of total pyrrolizidine alkaloids per day, one died on day 34 and the other survived until day 35 when it was painlessly killed. There were significant elevations in serum AST and GGT activities in these calves. The histological lesions of the calf surviving until 35 days were compatible with pyrrolizidine alkaloid toxicity, that is megalocytosis, karyomegaly and necrosis of hepatocytes with karyomegaly of biliary epithelium. The pyrrolizidine base present in *Cynoglossum officinale* (heliotridine) and its esters have a similar type of toxicity to the highly toxic and more familiar macrocyclic diester pyrrolizidine alkaloids of the pyrrolizidine base (retronecine), present in *Senecio* or *Crotalaria* species.

Introduction

Cynoglossum officinale (hound's-tongue) is a plant, native in Britain, Europe and Russia, which was introduced inadvertently in the United States. This plant has been reported to cause deaths of cattle in Britain and Russia, and is also suspected to have caused deaths of calves in the U.S. (Greatorrex, 1966; Mandryka, 1979; Baker, Smart, Ralphs and Molyneux, 1989). *Cynoglossum officinale* has been implicated in the death and hepatic injury of horses consuming contaminated hay (Knight, Kimberling, Stermitz and Roby, 1984). The plant is not palatable to cattle or horses under range conditions, but when dried and mixed with hay it is readily consumed. The toxic compounds within the plant are thought to be pyrrolizidine alkaloids, with four different alkaloids being identified (Mattocks, 1986). The type of pyrrolizidine alkaloids present in *Cynoglossum officinale* (a heliotridine-type necine ring) differs from the retronecine-type necine ring present in other more familiar pyrrolizidine alkaloid-containing plants (*Senecio*, *Crotalaria*, *Heliotropium*). Purified preparations of two of the pyrrolizidine alkaloids in *Cynoglossum officinale* (ethinatine, heliosupine) have been given to rats with heliosupine having one fifth the LD₅₀

of echinatine (Bull, Culvenor and Dick, 1968). There is little information regarding the relative toxicity of the heliotridine type of pyrrolizidine alkaloids in other animal species.

Intoxications of cattle by this plant to date have been limited to naturally occurring exposures, in which an unknown total plant or pyrrolizidine alkaloid dose was received by each animal, and there is little information regarding the type, degree or extent of tissue injury caused by the plant. Several of the reports have not included information regarding the cause of death or type of tissue injury (Greatorrex, 1966; Mandryka, 1979). Another report suggested that pyrrolizidine alkaloids caused hepatic failure in a calf with typical histological changes indicative of pyrrolizidine alkaloid toxicosis and *Cynoglossum officinale* was the only known available source of pyrrolizidine alkaloids (Baker *et al.*, 1989). Previous experimental feeding of the plant to a single pony resulted in no gross changes in the liver, or microscopic changes in liver architecture that were specific for pyrrolizidine alkaloid intoxication (Knight *et al.*, 1984). The purposes of this study were to: (1) substantiate that consumption of *Cynoglossum officinale* causes pyrrolizidine alkaloid-mediated hepatic injury in cattle and (2) determine its relative toxicity to cattle compared to that of other types of pyrrolizidine alkaloids.

Materials and Methods

Animals

Six, 6- to 8-month-old Hereford calves (mean body weight 153 ± 38 kg) were divided into two groups. Calves were vaccinated against IBR, PI₃ and BVD viruses and Clostridium toxins. Faecal samples were examined for evidence of trematode or nematode parasitism. All calves were allowed free access to salt blocks and water and were fed alfalfa hay twice daily ad libitum.

Plant Material

Two collections of *Cynoglossum officinale* were combined and given to the calves during this study. The collections near Thistle, Utah, were made in June 1985 and 1988 when the second year's growth was flowering or just past flowering. The two collections were air-dried, ground through a 2-mm screen and combined. The mixture was assayed for total pyrrolizidine alkaloid content by nuclear magnetic resonance, with heliosupine (the predominant alkaloid present) as the standard (Molyneux, Johnson, Roitman and Benson, 1979). The total pyrrolizidine alkaloid content on a dry weight basis was 0.73 per cent, with 0.18 per cent as the free base and 0.55 per cent in the N-oxide form.

Dosing

Calves were divided into two groups, (Group A, $n=4$ calves; Group B, $n=2$ calves) and each calf was given sufficient dried, ground plant material once daily to equal 60 mg per kg per day of total pyrrolizidine alkaloid (Group A) or 15 mg per kg per day of total pyrrolizidine alkaloid (Group B). The plant material was mixed with two parts of water into a slurry and given by stomach tube at 0800 hours each day. The average daily dose of plant material on a dry weight basis was 1072 and 412 g for group A and B calves, respectively. All animals were scheduled to be dosed for 21 days then destroyed for post-mortem examination on day 35 by administration of T-61® (Hoechst-Roussel, Sommerville, NJ, U.S.A.).

Sample Collection

Before starting *Cynoglossum officinale* administration, calves were allowed to adjust to the feed and pen environment for 7 days. Two blood samples, 3 to 4 days apart, and a liver biopsy sample were obtained prior to initiation of *Cynoglossum officinale* administration. Calves were bled twice weekly beginning on day 1 following initiation of plant administration. Blood was allowed to clot and serum was retained for determination of serum sodium (Na), potassium (K), chloride (Cl), glucose (Glu), urea nitrogen (BUN), creatinine (Cre), calcium (Ca), magnesium (Mg), phosphorus (P), total protein (TP), albumin (Alb), bile acid and total bilirubin (T Bili) concentrations. Serum alanine transaminase (ALT), aspartate transaminase (AST) and gamma-glutamyltransferase (GGT) activities were also assayed. Serum analyses for other than GGT activity and serum bile acid concentrations were done with an automated serum analyser (Monarch 2000 Chemistry System, Instrumentation Laboratories, Lexington, MA, U.S.A.). Serum GGT activity was determined with an automated batch analyser and prepared reagents (Abbott Biochromatic Analyser 200, Abbott Laboratories, Diagnostic Division, North Chicago, IL, U.S.A.). Serum bile acid concentration was determined with an automatic analyser and prepared reagents (Hitachi 704 Automatic Analyzer, Boehringer-Mannheim Diagnostics, Indianapolis, IN; Sigma, St. Louis, MO, U.S.A.). Plasma BSP clearance, t_1 , was determined prior to feeding, when feeding ceased and at the time of euthanasia with 0.5 g of BSP (Sigma, St. Louis, MO) and an automatic analyser (Abbott Biochromatic Analyser 200, Abbott Laboratories, Diagnostic Division, North Chicago, IL). Liver biopsy samples were obtained before feeding *Cynoglossum officinale* and once every 7 days during feeding with a Vim Silverman true cut biopsy needle between the 9th and 10th rib approximately one quarter of the distance from the dorsal spinus process to the sternum. Liver samples were fixed by immersion in neutral buffered 10 per cent formalin. Surviving calves were humanely killed at the end of 35 days as described above. Samples of liver, lung, kidney, brain, pancreas, intestine, rumen, adrenal gland and heart were collected and fixed by immersion in neutral buffered 10 per cent formalin for histological examination.

Changes in serum chemistry were evaluated by one-way analysis of variance and Duncan's multiple range test for significant changes between before and after initiation of *Cynoglossum officinale* feeding.

Results

All calves in group A died within 48 h of a single administration of plant material. One of the two calves in Group B died 13 days after the 21 day feeding schedule was completed, and the other was destroyed 14 days after the 21 day feeding schedule was completed.

Group A

One calf was unable to stand 24 h after the first administration of plant and the remaining calves had difficulty in moving. Two calves died 36 h after the initial administration and the remaining two calves died 48 h after administration of *Cynoglossum officinale*.

There was a significant ($P < 0.05$) eight- to ten-fold elevation of serum GGT activity, bilirubin and bile acid (Fig. 1) concentrations and a 30 fold elevation of serum AST activity 24 h after the initial administration of dried plant material. There was a significant ($P < 0.05$) decrease in the serum glucose (to a mean of 11 mg per dl) and total protein concentration (decrease of 0.5 g per dl)

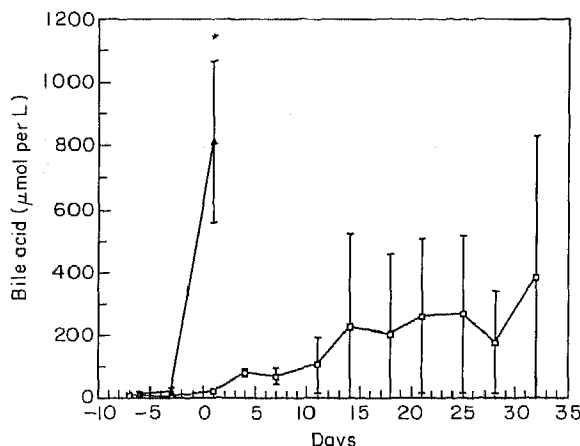


Fig. 1. Mean serum bile acid concentration ($\mu\text{mol per l}$) in serum of calves given 60 mg per kg per day (\blacktriangle) or 15 mg per kg per day (\square) of total pyrrolizidine alkaloid in dried, ground *Cynoglossum officinale*. *Indicates mean value is significantly ($P < 0.05$) different from values before administration of the plant.

24 h after the initial administration of dried plant material. No other serum measurements were significantly changed from pretreatment nor did they fall outside the reference range for cattle. Post-feeding BSP $t_{\frac{1}{2}}$ times were not determined for group A calves, but were within reference ranges prior to *Cynoglossum officinale* feeding.

Gross post-mortem findings in the calves were similar. All calves had swollen, blood filled livers and segmental congestion and haemorrhage of the intestinal mucosa that was most severe in the duodenum. Three of the calves had serosal petechial haemorrhages and oedema of the mesentery surrounding the pancreas and duodenum. Two calves also had subcutaneous oedema, especially of the brisket region.

The most striking histological lesion in all calves was massive hepatocellular necrosis, with occasional viable centrilobular hepatocytes or randomly scattered hepatocytes throughout the lobule (Fig. 2). Kupffer cells often contained erythrocytes within their cytoplasm. Additionally, there was subendocardial and interstitial haemorrhage in the myocardium. Splenic peri-arteriolar lymphoid sheaths and intestinal lymphoid follicles had necrosis of lymphocytes with pyknotic nuclei and karyorrhectic nuclear debris present in follicles. Other lesions were inconsistent and included mild enteritis, oedema of the gall bladder mucosa and mild bronchopneumonia.

Group B

Serum changes were not as dramatic as those of Group A calves, but there was a significant elevation of serum GGT (five-fold on days 28 and 32) and AST (three-fold on days 4 and 7) activities and serum total protein concentration (7.8 g per dl on day 28). Serum bile acid concentration was increased (Fig. 1), but the response of the two calves was variable, with the calf that died having

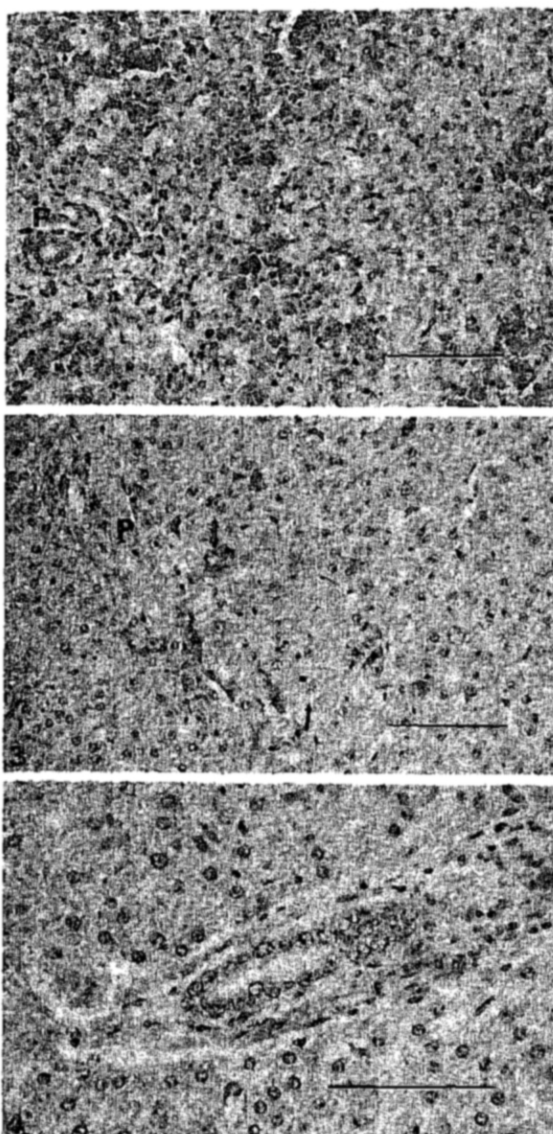


Fig. 2. Photomicrograph of liver of a calf given a single dose of dried, ground *Cynoglossum officinale* plant material equivalent to 60 mg per kg of total pyrrolizidine alkaloid. Periportal and midzonal hepatocytes are necrotic with pyknotic cellular debris remaining. Centrilobular hepatocytes remain. Central vein (C) and portal regions (P) are indicated. Bar = 100 μ m. HE.

Fig. 3. Photomicrograph of liver of a calf given dried, ground *Cynoglossum officinale* plant material equivalent to 15 mg per kg per day of total pyrrolizidine alkaloid for 21 days and examined on day 35. A portal region (P) is indicated. Random hepatocytes are necrotic (arrowhead) with anisokaryosis and megalocytosis (arrow). Bar = 100 μ m. HE.

Fig. 4. Photomicrograph of liver of a calf given dried, ground *Cynoglossum officinale* plant material equivalent to 15 mg per kg per day of total pyrrolizidine alkaloid for 21 days and examined on day 35. A portal region with anisokaryosis of biliary epithelial nuclei. Bar = 100 μ m. HE.

greater increases. Serum Alb concentration was significantly ($P < 0.05$) decreased on two days, but the decrease was not outside the reference range for cattle. Serum Glu and BUN concentrations tended to decrease later in the feeding, but the changes were not consistent or significant. Pre-feeding BSP t_1 times were within the reference range for cattle (< 4 min) and were prolonged at the end of feeding at 21 days (11.5 min and 23 min) and at the time of euthanasia (10.5 min).

The calf that died the day before the last feeding in group B was too autolysed to examine tissues histologically. Tissues from the calf that was killed exhibited anisokaryosis and karyomegaly of hepatocyte, biliary epithelial (Figs 3 and 4) and renal tubular epithelial nuclei. Additionally, there was occasional megalocytosis of hepatocytes, with individual, random, hepatocellular necrosis throughout the liver (Fig. 3).

Discussion

The two groups of cattle exhibited different responses to the amounts of *Cynoglossum officinale* given. This reflected the total dose of pyrrolizidine alkaloid and the time over which it was administered to each group of animals. Doses of pyrrolizidine alkaloids are cumulative within the liver, but the duration of exposure to similar doses will result in different responses. Pyrrolizidine alkaloids are metabolized by hepatocyte mixed function oxidase systems to pyrroles, that bind to DNA and cytosolic proteins, and also inhibit protein synthesis, cellular division and cellular respiration (Christie and LePage, 1962; Thorpe and Ford, 1968; Villa-Trevino and Leaver, 1968; Mattocks and White, 1971; Mattocks, 1985). Long term exposure to a low dose of pyrrolizidine alkaloids will cause cirrhosis, megalocytosis, karyomegaly, bile duct hyperplasia and fibrosis of central veins and portal areas (Hill and Martin, 1958; Jago, 1969). The same or smaller dose of pyrrolizidine alkaloids administered in a short period of time will cause massive haemorrhagic, hepatocellular necrosis and acute hepatic failure (Mattocks, 1986).

All Group A calves exhibited massive, acute, haemorrhagic hepatocellular necrosis and rapid death after a single dose of *Cynoglossum officinale*. The serum biochemical changes in these calves support a diagnosis of acute hepatocellular necrosis and loss of most of the functional hepatic mass. There was severe hypoglycaemia in conjunction with marked increase of serum AST and GGT activities and bile acid and bilirubin concentrations. Marked hypoglycaemia is associated with loss of hepatic mass and gluconeogenesis, while elevated AST, GGT and bile acid are compatible with increased hepatocellular release owing to damage and decreased serum clearance (Leifer and Peterson, 1984; Angsubhakorn, Poomvises, Romruen and Newberne, 1981; Duncan and Prasse, 1986). Acute hepatocellular loss and necrosis was substantiated on histological examination of the liver. This type of acute, massive hepatocellular necrosis has been reported previously after administration of high doses of pyrrolizidine alkaloids (Mattocks, 1986). Group B calves were exposed to greater total amounts of pyrrolizidine alkaloids than the calves in Group A. However, there was gradual but continual loss of hepatocytes and the development of more

characteristic lesions of megalocytosis and karyomegaly. Elevated bile acid concentration has been suggested to indicate a poor prognosis for survival in horses intoxicated by *Senecio* sp. pyrrolizidine alkaloids (Mendel, Witt, Gitchell, Gribble, Rogers, Segall and Knight, 1988). The prolonged BSP $t_{\frac{1}{2}}$ clearance indicated reduced hepatocellular mass in both calves of group B.

The alkaloids of *Cynoglossum officinale* are esters of heliotridine (Mattocks, 1986). Toxicity studies in rats indicate that macrocyclic diesters of retronecine are the most toxic, followed by open chain diesters of retronecine and heliotridine, then otonecine diesters and finally monoesters of heliotridine, retronecine and supinine (Mattocks, 1986). Comparative toxicity data in cattle are not readily available, but when *Senecio longilobus* and *Senecio riddellii* were administered to cattle in amounts of plant material equivalent to 10 to 13 mg per kg and 15 to 20 mg per kg, respectively, of total pyrrolizidine alkaloids per day, death occurred within 3 to 31 days (Johnson and Molyneux, 1984; Johnson, Molyneux and Stuart, 1985). *Senecio riddellii* contains a single macrocyclic diester of retronecine, riddelline, which is present in the plant as both free base and N-oxide form, whereas *S. longilobus* contains a mixture of four such alkaloids. The alkaloids of *Cynoglossum officinale* caused the death of a calf at similar doses to the macrocyclic diesters of *Senecio riddellii* and may be nearly as toxic as riddelline in single alkaloid preparations.

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